Figure 1

Schematic representation of human tenascin-C and of the strategy followed for the generation of BC2-like antibodies

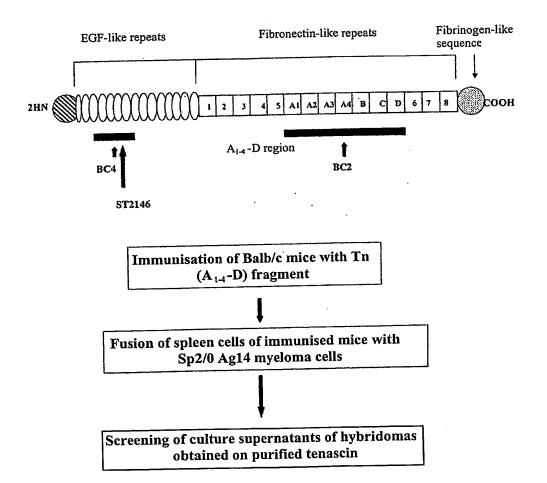
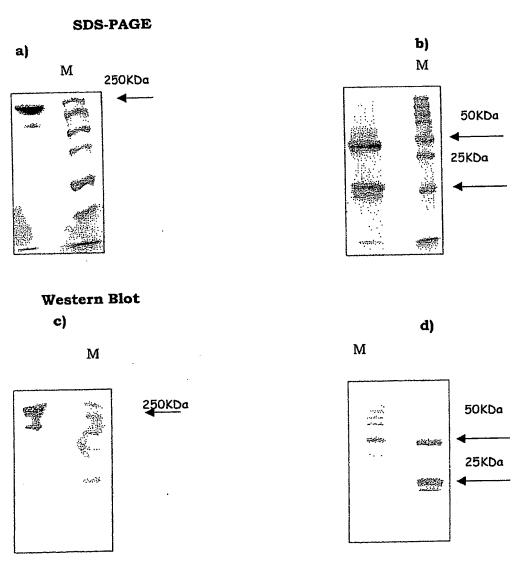


Figure 2

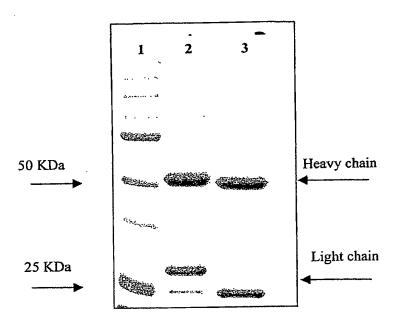
SDS-PAGE and Western Blot analysis of ST2485 antibody, in reducing (b, d) and non-reducing (a, c) conditions.



M: molecular weight standards

Figure 3

ST2485 antibody digestion with Flavobacterium Peptide-N-glycosidase enzyme (PNGase F).



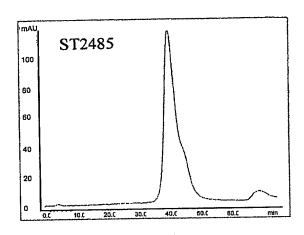
1: Molecular weight marker

2: Non-digested ST2485

3: PNGaseF-digested ST2485

Figure 4

BC-2 and ST2485 antitenascin antibodies hydroxyapatite chromatography.



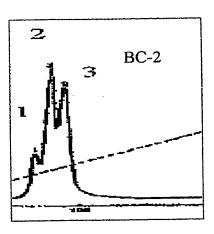
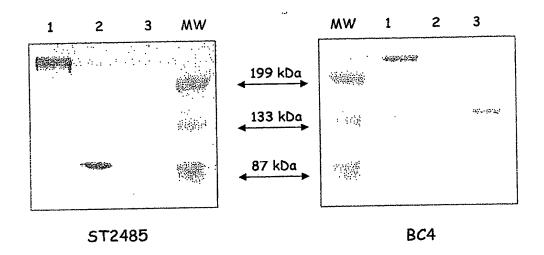


Figure 5

ST2485 antitenascin antibody Western Blot Analysis



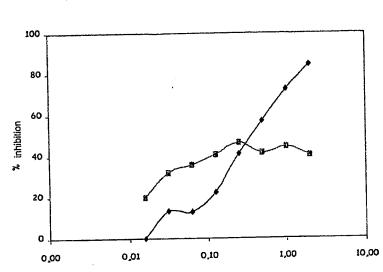
- 1: Tenascin-C
- 2: Tn A(1-4)-D Fragment
- 3: Tenascin EGF-like region recombinant fragment, containing the epitope recognized by BC-4 antibody.

MW: molecular weight standards

Figure 6

Competitive ELISA test between ST2485 and BC-2 for antigen binding.

a)



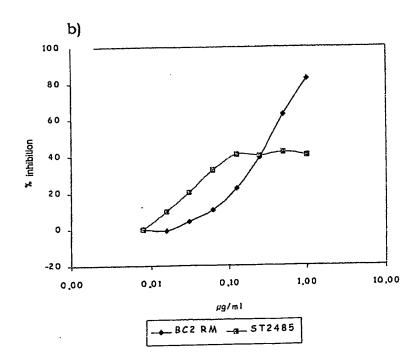
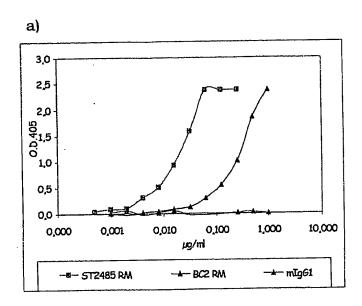


Figure 7

Immunoreactivity of ST2485 antibody in comparison with BC-2, on tenascin C (a) and on Tn $A_{(1-4)}$ -D fragment (b).



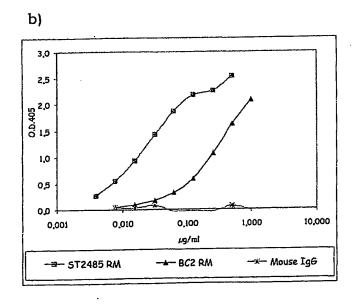
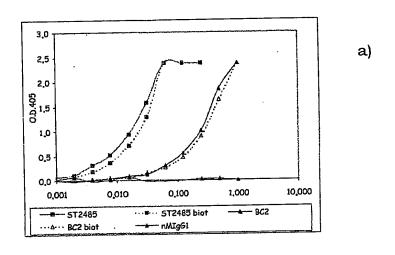
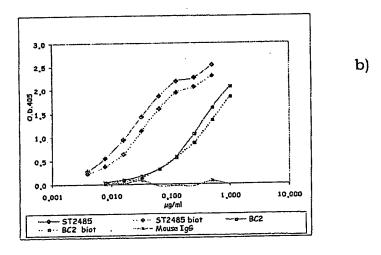


Figure 8

Immunoreactivity of ST2485 and BC-2 biotinylated and non-biotinylated antibodies, on tenascin-C (a) and on Tn $A_{(1-4)}$ -D fragment (b).





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Figure 9

Cross-reactivity of ST2485 antibody with murine tenascin.

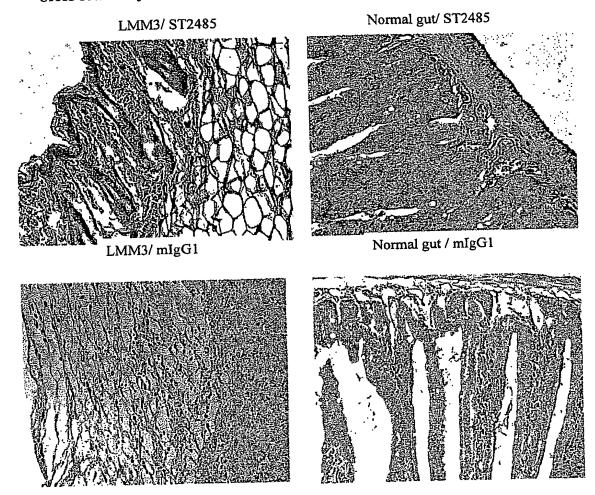
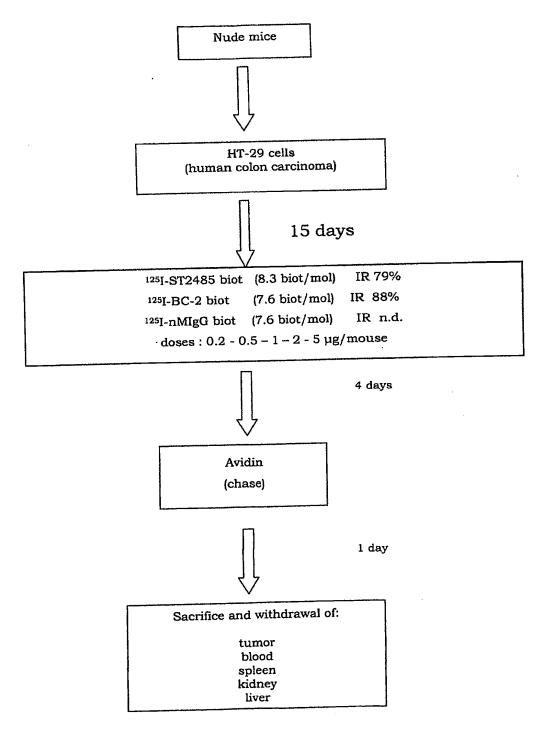


Figure 10

Biotinylated ST2485 and BC-2 antibodies biodistribution study protocol in human tenascin-expressing tumor-implanted nude mice.

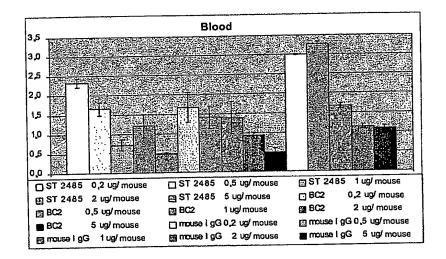


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Figure 11

Biotinylated ST2485 and BC-2 biodistribution in human tenascinexpressing tumor-transplanted nude mice. The antibody amount is expressed as percent of the injected dose per tissue gram (% I.D./gr).



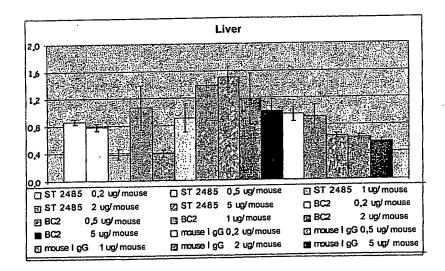
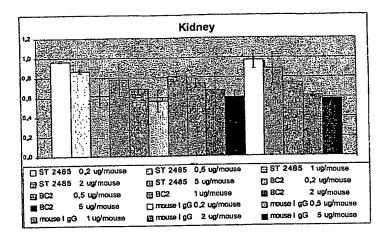
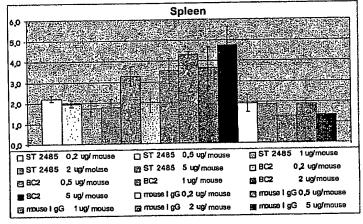


Figure 11a





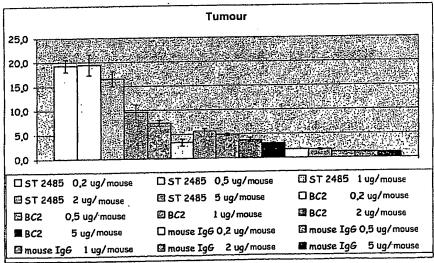
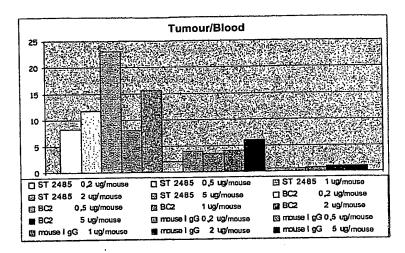


Figure 12

Biotinylated ST2485 and BC-2 biodistribution in nude mice: tumor/non tumor ratio.



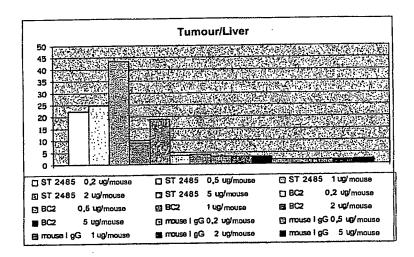
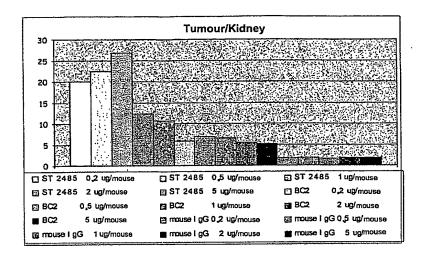


Figure 12a



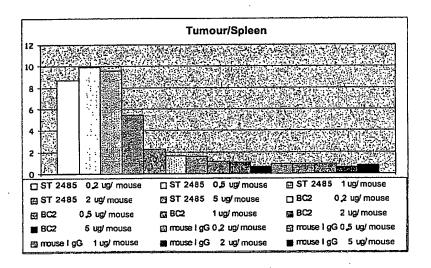
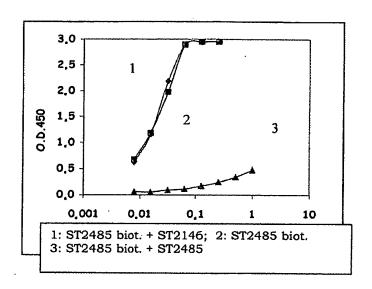


Figure 13

Interference (a) and additivity (b) ST2485 and ST2146 antitenascin antibodies in vitro evaluation by ELISA test.

a) Interference



b) Additivity

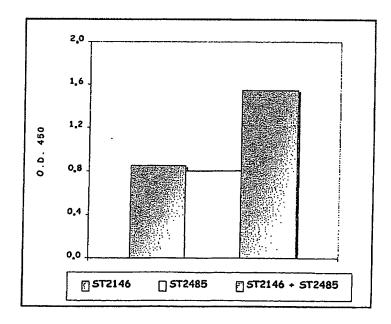
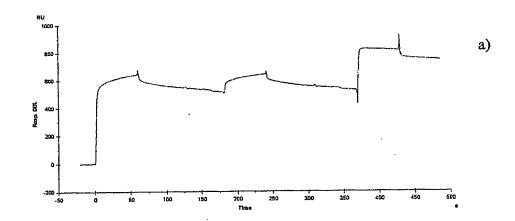


Figure 14

Antibodies ST2485 and ST2146 tenascin binding in vitro additivity by BIACore



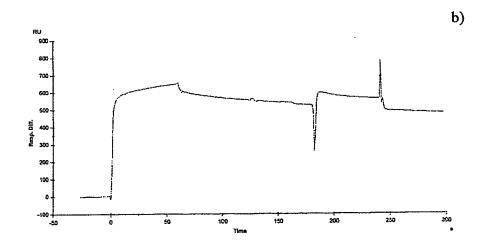
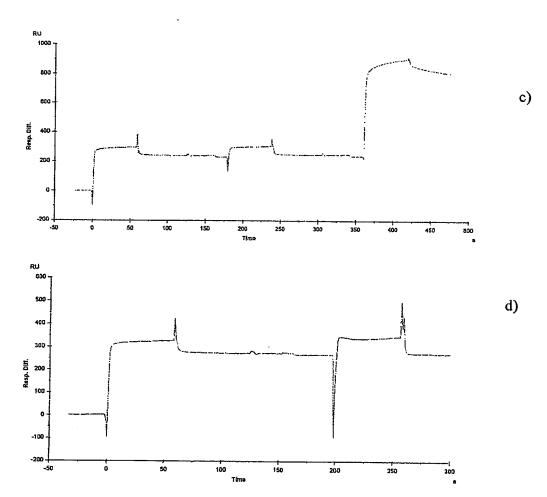


Figure 14a



i,

Figure 15

Schematic representation of in vivo additivity study in animal model

Tenascin-expressing human colon carcinoma (HT29)-transplanted nude mice Single or admixed radiolabelled biotinylated antibodies i.v. administration, 2ug/mouse dose each: 125[-ST2485 125I-ST2146 125I-ST2485 + 125I-ST2146 125I-ST2485+ ST2146 125I-ST2146+ ST2485 ¹²⁵I-ST2485+ nMIgG ¹²⁵I-ST2146+ nMIgG 125I-nMlgG \Box 6 days avidin i.v. administration at a dose 100x with respect to antibody (chase) П 1 day Sacrifice and withdrawal of: tumor, blood, spleen, kidney, liver

Figure 16

ST2485 e ST2146 antibodies additivity in animal model; tumor seat localization.

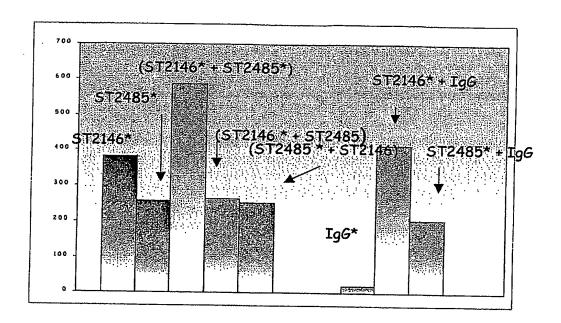


Figure 17

SEQID 1 sequence of ST2485 kappa light chain variable region (VL).

Signal peptide

ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTGCTTCAGTCATAATGTCCAGAGGACAAA Met Asp Phe Gin Val Gin lie Phe Ser Phe Lea Lea Ile Ser Ala Ser Val Ile Met Ser Arg Gly Gin

TTGTTCTCCCAGTCTCCAGCAATCCTGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGC He Val Leu Ser Gin Ser Pro Ala He Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys

N-glycosylation

CDR 1
AGGGCCAACTCAAGTGTACGTTTCATGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACC Arg Ala Ash Ser Ser Val Arg Phe Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys

CDR2
CTGGATTTATECAASCTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGG
Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly

GACCTCTTATTCTGTCACAATCAGCAGAGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGC Ser Gly The Ser Tyr Ser Val The Ile Ser Arg Val Glu Ala Glu Asp Ala Ala The Tyr Tyr Cys Gln

ALTO CONTROL OF THE TWO TO THE TOTAL CONTROL OF THE TOTAL CONTROL OT THE TOTAL CONTROL OF THE TOTAL CONTROL OF THE TOTAL CONTROL OT THE Gin Trp Ser Ser Ash Ser Pro Arg Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Arg Arg Ala

Figure 18

SEQID 2 sequence of ST2485 gamma heavy chain variable region (VH)

Signal peptide

ATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGAACTGCAGGTGTCCACTCTGAGGTCCAGCTG
Met Glv Trp. Ser Trp lle Phe Leu Phe Leu Leu Ser Glv Thr Ala Glv Vol His Ser Glu Val Gln Leu

CAACAGTCTGGACCTGAGCTGGAAGCCTGGAGCTTCAATGAAGATTTCCTGCAAGGCTTCTGG Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala Ser Mei Lys lle Ser Cys Lys Ala Ser

TTACTCATTCACTGGGTGAACGGGTGAAGCAGCCATGGAAAGAACCTTGAATGGA Gly Tyr Ser Phe Thr Gly Tyr Thr Met Asn Trp Val Lys Gln Ser His Gly Lys Asn Leu Glu Trp

TTGGACTTENTALEGEGACAATGGTGGTACTACCTACAACCAGAAGTTCAAGGGCAAGGCCACA lle Gly Leu fle Asn Pro His Asn Gly Gly Thr Thr Tyr Asn Gln Lys Phc Lys Gly Lys Ala Thr

TTAACTGTAGACAAGTCATCCAACACACACCCTACATGGAGCTCCTCAGTCTGACATCTGAGGACTC Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr Met Glu Leu Leu Ser Leu Thr Ser Glu Asp

TGCAGTCTATTACTGTACAAGAGGGGGGGGGTTACTACTGGTTCTTCGATGTCTGGGGCGCAGGGA Ser Ala Val Tyr Tyr Cys Thr Arg Pro Gly Gly Tyr Tyr Trp Phe Phe Asp Val Trp Gly Ala Gly

CCACGGTCACCGTCTCCTCA The The Val The Val Ser Ser